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Interactive effects of temperature, pH, and antibiotics reshape zebrafish physiological stability

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INTRODUCTION

Aquatic ecosystems are increasingly **subjected** to **multiple stressors**;

Antibiotics

- **Sulfamethoxazole** (SMX), **Trimethoprim** (TRIM) are two of the **most used antibiotics** in the last 50 years;
- These antibiotics and **their mixture** (MIX) are **widely used and frequently detected** in **aquatic ecosystems**[1];

Climate change

- **Climate change** drives variations in key environmental factors, like **temperature** and **pH**;
- **These changes** can **alter how available** and **harmful antibiotics** are, **affecting aquatic organism** and possibly **ecosystem health**;

Understanding how these **stressors interact is essential for assessing the risks to biodiversity and ecosystem functioning**, reinforcing the importance of **integrative ecotoxicological approaches** in **environmental risk assessment**.

AIM

Effects of **SMX** (150 µg/L), **TRIM** (30 µg/L), and **MIX** (150 µg SMX/L + 30 µg TRIM/L), under two environmental scenarios: **standard** (26 °C + pH 7.5) and a **climate-change scenario** (28 °C + pH 9.0) were evaluated, using an integrated multi-biomarker approach to assess the organism's biological health status.

METHODS

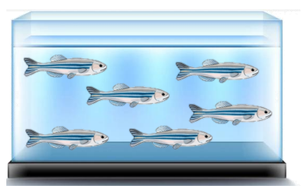
Chronic assay: OECD 2000 Test Nº 215[2]

Danio rerio juveniles

Antibiotics

Sulfamethoxazole (150 µg SMX/L) | **Trimethoprim** (30 µg TRIM/L) | **Mixture** (150 µg SMX/L + 30 µg TRIM/L)

Concentrations selected based on environmentally relevant concentration[1]



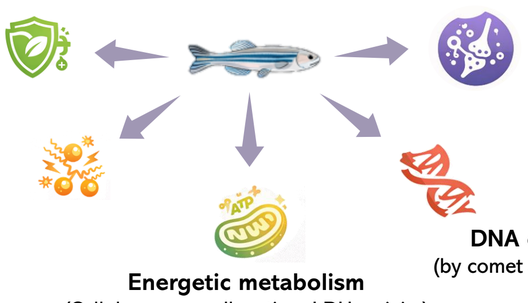
Standard scenario

26º C | pH 7.5 | Standard temperature and pH according to OECD 2000[2]

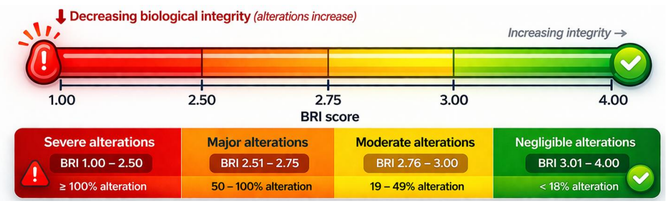
Climate-changed scenario

28º C | pH 9.0 | Warming and alkalinization scenarios[3,4]

After 28 days of exposure

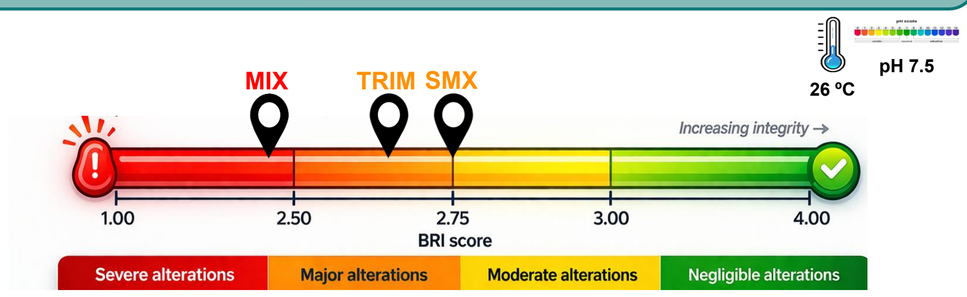


Integrated biological health status of *Danio rerio* based on Biomarker Response Index (BRI)[4,5]



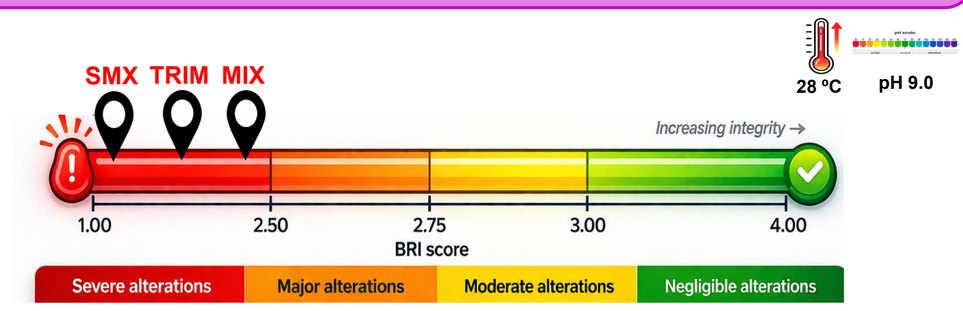
RESULTS

Standard scenario



- **Environmentally relevant concentrations** of **SMX**, **TRIM**, and **MIX** induced **physiological disruptions** in *D. rerio;*
- **SMX** and **TRIM** caused **major alterations**, mainly associated with alterations in *D. rerio* antioxidant/detoxification responses, and energy metabolism;
- **MIX induced the strongest toxicity**, causing **severe alterations**, impairing *D. rerio* health (e.g., lipid peroxidation, and DNA damage).

Climate-changed scenario



- **All treatments** induced **severe alterations** in *D. rerio*, including lipid peroxidation, DNA damage, and disruption of energy metabolism, indicating a **clear loss of physiological stability;**
- The small differences in observed effects may suggest an **interactive effect** within the MIX, where combined exposure **triggers biological responses** that slightly reduce toxicity compared to individual exposures, possibly through **the activation of compensatory or adaptive mechanisms**;

CONCLUSIONS

- **Antibiotics** have **inherent toxicity**, disrupting fish physiological homeostasis **even without** other **environmental factors;**
- **Environmental factors** can **modulate antibiotic toxicity** by affecting chemical bioavailability, membrane permeability, and enzymatic activity, thereby **influencing overall toxicological outcomes;**
- **Warming** and **alkalinization enhanced antibiotic toxicity**, both individually and in mixtures, resulting in **more pronounced adverse effects** on ***D. rerio***;
- These findings highlight the need for **integrative studies** that simultaneously address **chemical pollution** and **climate-related stressors** to improve **ecological risk assessment under realistic multi-stressor scenarios**;

REFERENCES

1. Diogo, B.S., Rodrigues, S., Antunes, S.C., 2025. Mixture matters: Exploring the overlooked toxicity of sulfamethoxazole and trimethoprim in aquatic environments. Environmental Toxicology. https://doi.org/10.1002/tox.24528;
2. OECD, 2000. Test No. 215: Fish, Juvenile Growth Test. https://doi.org/10.1787/20745761;
3. Diogo, B.S., Speksnijder, B., Rodrigues, S., Golovko, O., Antunes, S.C., 2025. Chronic toxicity of antibiotics and global warming in *Danio rerio*: Biomarker responses and toxicological effects. Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology. https://doi.org/10.1016/j.cbpc.2025.110240;
4. Diogo BS, Rodrigues S, Golovko O, Antunes SC. 2025. Dual Environmental Stressors: How pH modulates antibiotic toxicity in *Danio rerio*? Environmental Toxicology and Pharmacology. https://doi.org/10.1016/j.etap.2025.104774
5. Li, X., Wang, M., Chen, W., Jiang, R., 2019. Evaluation of combined toxicity of Siduron and cadmium on earthworm (*Eisenia fetida*) using Biomarker Response Index. Science of The Total Environment 646, 893–901.10. https://doi.org/1016/j.scitotenv.2018.07.380;

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